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(71) Applicant: GENETIC SYSTEMS CORPORATION [US/US]; 3005 First Avenue, Seattle, WA 98121 (US).

(72) Inventors: HOFFMAN, Allan, S. ; 4528 West Laurel Drive N.E., Seattle, WA 98105 (US). MONJI, Nobuo ; 3545 N.E. 98th Street, Seattle, WA 98115 (US).

(74) Agents: MAKI, David, J. et al.; Seed and Berry, 6300 Columbia Center, Seattle, WA 98104-7092 (US).

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(54) Title: METHODS FOR SELECTIVELY REACTING LIGANDS IMMOBILIZED WITHIN A TEMPERATURE-SENSITIVE POLYMER GEL

(57) Abstract

Methods for delivering substances into, removing substances from, or reacting substances with a selected environment utilizing polymer gels or coatings characterized by a critical solution temperature (CST). The CST as well as the pore structure, pore size, pore distribution, and absorbing capacity of the gel may be selectively controlled. The substances may be physically or chemically immobilized within the polymer gels. In addition, a method for altering the surface wettability of CST polymers is also disclosed.

1

Description

"METHODS FOR SELECTIVELY REACTING LIGANDS
IMMOBILIZED WITHIN A TEMPERATURE-SENSITIVE POLYMER GEL"

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. application Serial No. 853,697, filed April 17, 1986 and assigned to Genetic Systems Corporation.

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Technical Field

The present invention relates generally to methods for delivering substances into, removing substances from, or reacting substances with a selected environment in order 10 to effect a desired purpose, utilizing polymer gels or coatings exhibiting either an upper or a lower critical solution temperature.

Background Art

15   A number of polymers undergo a phase separation in response to a change in environmental conditions, such as solution pH, ionic strength or temperature. For instance, some soluble polymers become insoluble when the solution temperature is changed only a few degrees. These 20 polymers are said to possess a critical solution temperature (CST). A polymer possessing a lower critical solution temperature (LCST) becomes insoluble when the temperature of the solution is increased through a particular narrow temperature range. Conversely, a polymer possessing an 25 upper critical solution temperature (UCST) becomes insoluble when the temperature of the solution is decreased through a particular narrow temperature range.

Polymers capable of phase changes in response to temperature changes have been described by Taylor in U.S. 30 3,427,892 for use in controlling a process relating to film developing. In Taylor, a layer of polymer changes permeability as a function of temperature. The polymers are

mammalian body, for example. The gel is said to release
insulin into the body over time as it diffuses through the
gel pores.

5   Cussler, in U. S. Patent No. 4,555,344, describes
using a cross-linked ionic polymer gel, such as partially
hydrolyzed polyacrylamide or dextran, to selectively absorb
a low molecular weight solvent and solute from a solution
that includes higher molecular weight components in the
solution.   The gel is introduced into the solution in a
10   shrunken state.   A pH change or a change in composition of
the solution is required to cause the gel to rapidly swell
in volume, absorbing low-molecular weight solvent and
solute.

Graham, in U. S. Patent No. 4,584,188, describes
15   gels comprising a polymerizable cyclic (thio) ether and a
hydrophilic homo- or copolymer.   A temperature change is
required for expelling or releasing from the gel an active
substance previously absorbed from a solution.

A limitation to date regarding the use of polymer
20   gels that change phases in response to a change in environ-
mental conditions has been that separations from or deliv-
eries to solutions have been substantially nonspecific.
Both the Cussler and the Graham inventions rely upon a
temporary physical entrapment of the solution or solvent
25   within the gel.   That is, a CST gel, for example, in
response to a change in temperature through the CST absorbs
a liquid to which it has been exposed.   The solution is
absorbed by the gel nonspecifically, only excluding mole-
cules too large for its pore structure.

30   Consequently, there exists a need in the art for
an improved system for controlling biological or chemical
reactions in selected environments by providing methods of
separating certain desired substances from a solution,
delivering certain selected substances to, or exposing
35   certain selected substances to a desired environment, which
methods are readily and efficiently controllable.   The

with the substance desired to be delivered to the selected
environment.   The temperature of the polymer gel is
adjusted, whereby the gel absorbs the substance.   The gel
is placed into contact with the environment of interest and
5   the temperature adjusted, whereby the gel releases the sub-
stance into the environment.   As noted above, the initial
temperature of the polymer gel may be such that it is in a
partially or totally desolvated state when contacted with
the substance to be absorbed and subsequently delivered.
10 Alternatively, the polymer may be in a solvated state,
contacted with the substance, and the temperature cycled,
causing the polymer to desolvate and, upon reversing the
temperature, to solvate, absorbing the desired substance.

      The substance may be incorporated in the polymer
15 by physical absorption or entrapment within the pores of
the gel.   The substance may also be incorporated or
entrapped via molecular entanglements and interactions
within denser regions of the polymer gel, typically by
means of secondary (ionic, polar, hydrophobic, etc.) forces
20 binding the substance to the polymer.   In addition, the
substance may also be bound to the gel matrix by means of
labile, primary covalent bonds.   A labile bond arrangement
is useful in drug delivery, wherein, for example, an LCST
gel polymer at 37°C is desolvated and includes the drug
25 bound to the polymer by means of the labile bond.   The gel
is injected into the body in the form of gel particles or
microspheres including the drug.   The desired delivery area
is then cooled to 35°C, which causes the gel to swell and
absorb surrounding aqueous solution, whereupon the drug
30 polymer bond is broken and the drug released.

      The pore structure of the CST polymer may be
adjusted by a number of means to selectively retain a
desired substance within the gel.   Pore structure, and
hence, the amount of substance released and release rate
35 are selectively controlled by adjusting the composition
and/or concentration of monomers in the synthesis mixture
employed to form the gel.   Also, adjusting the amount and

tially hydrophobic N-substituted acrylamides or methacrylamides), hydroxy alkyl celluloses, polyoxazolidones, polyvinylmethylether, polyethylene oxide, polymethacrylic acid, or copolymers thereof, including
5   variations in the polar or hydrophobic components of all of these polymers.

The methods described herein may also include a polymer gel that is characterized by an upper critical solution temperature (UCST). Such a polymer gel may include a
10   polymer such as polyacrylic acid, polymethacrylamide, or polyvinyl alcohol, and copolymers thereof.

The methods described herein, in addition to delivery, allow the separation of a desired substance from a solution. Typically, the procedure is initiated by intro-
15   ducing a dry or shrunken polymer gel characterized by a critical solution temperature (as described above) into the solution containing the desired substance. Utilizing the CST character of the polymer, one can adjust the temperature of the gel/solution sufficiently to cause the gel to
20   swell and absorb the desired substance. As with the delivery method, the process may utilize either a lower or upper critical solution temperature polymer of the type described above.

This separation method is particularly useful in
25   bioseparations, wherein the polymer gel includes a first component of a binding pair bound physically or chemically to the polymer. The polymer gel/binding component is contacted with a solution that contains the second component of the binding pair. This mixture is then incubated at a
30   temperature sufficient to cause the polymer gel/first binding component to absorb liquid from the solution containing the second binding component, thereby allowing the second binding component to specifically bind to the first binding component. The solution is then heated or cooled to cause
35   the gel to shrink and desolvate whereby the gel releases the remaining solution and retains the bound binding pairs.

ciently to allow solution containing the enzyme substrate
from the environment of interest to contact the enzyme,
thereby initiating and catalyzing the reaction of interest.
Further adjustment of the temperature allows one to control
5   the rate of reaction.   Reversing the temperature beyond a
CST will cause the polymer gel to shrink, closing off
contact of the reactant with the solution, thereby substan-
tially slowing down or terminating the reaction.   Alterna-
tively, the reaction may be selectively controlled by
10   gradually causing the polymer gel to shrink.   In addition,
through use of a combination of temperature and controlled
pore size, one can selectively eliminate access of differ-
ent sized molecules as the temperature is raised or
lowered.

15   These and other aspects of the present invention
will become evident upon reference to the following
detailed description and attached drawings.

Brief Description of the Drawings

20   Figure 1 presents vitamin B_{12} delivery as a func-
tion of cross-linker/monomer ratio, for indicated solution,
monomer and solvent composition used to synthesize poly-
NIPAAm gels.

            Figure 2 depicts myoglobin delivery as a function
25   of cross-linker/monomer ratio for various NIPAAm polymer
gels synthesized by the indicated solution, monomer and
solvent compositions.

            Figure 3 illustrates the delivery of vitamin B_{12}
as a function of time for three different polyNIPAAm gels.

30   Figure 4 demonstrates the impact on water
contents vs. temperature of copolymerizing methacrylic acid
with NiPAAM to form LCST polymer gels.

            Figures 5 (a-c) depict the release of methylene
blue at 20°C and 50°C as a function of time for NIPAAm
35   polymer gels copolymerized with up to 5% methacrylic acid.

            Figure 6 depicts the release rate, as a function
of temperature, of methylene blue from a cross-linked

Best Mode for Carrying Out the Invention

        The methods of the present invention utilize, as
a principal component, a polymer that is characterized by a
capability of precipitating from an aqueous solution within
5 a narrow temperature range. The temperature at which
polymer precipitation is initiated is often termed the
"critical solution temperature" (CST) for the polymer.

        The CST polymer of interest may be copolymerized
and/or cross-linked to form a gel which absorbs or releases
10 a liquid or vapor in response to a temperature change at
the CST.

        Polymers having a CST are well known in the liter-
ature. See, for example, Molyneux, Water-Soluble Synthetic
Polymers; Properties and Behavior, CRC Press, Boca Raton,
15 Florida (1983); Finch, C. A., ed., Chemistry and Technology
of Water-Soluble Polymers, Plenum Press, New York (1983) at
163. A combination of hydrophobic and hydrophilic compo-
nents may be selected to form a desired CST characteristic.

        The majority of CST polymers exhibit phase separa-
20 tion or precipitation upon cooling and are referred to as
including an upper critical solution temperature (UCST)
characteristic. A number of polymers of interest, however,
exhibit precipitation upon heating. The temperature at
which precipitation occurs is referred to as the lower
25 critical solution temperature (LCST) of the polymer.

        Polymers having upper critical solution tempera-
tures (UCST) include many diverse polymers. A few aqueous
systems include polyacrylic acid, polymethacrylamide and
polyvinyl alcohol. Polymers that exhibit a lower critical
30 solution temperature (LCST) include polyethylene oxide,
polyvinylalkylethers, polyvinylmethyl oxazolidone, poly-
methacrylic acid, substantially hydrophobic N-substituted
acrylamide polymers, hydroxy alkyl celluloses as well as
alkylcelluloses. See Finch, C. A., ed., Chemistry and
35 Technology of Water-Soluble Polymers, Plenum Press, New
York (1983) at 157.

13

character, likewise may have a major impact upon gel pore structure as well as LCST or UCST.

Careful choice of ingredients and the synthesis parameters noted above allow production of a gel that is
5   capable of selectively retaining or excluding molecules on the basis of size. Relatively lower levels of cross-linking agent for a given monomer will result in larger pore sizes. Where self-supporting gels are desired, however, pore size increases effectuated by means of reducing cross-
10 linker are limited by the mechanical strength the gel must retain in order to hold together. For larger pore sizes with the same monomers, the gel may be incorporated within (another polymer or), attached to or applied as a coating on a supporting surface or base. The polymer gel coating
15 could be attached chemically or by means of plasma dis- charge, ionizing radiation, UV process, ozone process or the like. The gel may be cross-linked during or after processing using a variety of means to effect the cross- linking, including incorporation of cross-linkers during
20 the coating or incorporating process as well as the use of cross-linking compounds, ionizing radiation or UV after the system is polymerized.

The selection of cross-linking agents is one of the determining factors in achieving a desired gel pore
25 size, which will be important for larger substrates or other reactants. The cross-linker may be hydrophobic or hydrophilic. The hydrophobic cross-linker may be any short di- or trivinyl or di- or triallyl monomer, such as methy- lene bis-acrylamide. A hydrophilic cross-linking agent may
30 be, for example, a long-chain polyethylene glycol, both ends of which include double bonds for binding to the poly- mer backbone. Thus, the choice of cross-linker permits covalently bonding into the polymer gel hydrophobic or hydrophilic groups, which will effect the pore size and its
35 distribution.

The concentration and composition of the initi- ators used in a free-radical polymerization can also

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over which the separation (absorption) or delivery (desorption) occurs.   Such an extended response may be desired, depending upon the particular environment of interest.

Including a second CST polymer in the gel polymer-
5   ization mixture produces a gel which has two CSTs, each reflecting the character of its backbone polymer composition.   An interpenetrating network (IPN) of the two polymers is formed.   Thus, water or solution evolution from a gel in a delivery process for such an IPN gel will show
10 two distinct drops in swelling solution content as temperature is increased through the first and then the second LCST.

As noted above, the present invention allows the immobilization of a ligand within the gel in a manner such
15 that the ligand may be exposed to and/or isolated from an environment through the gel's CST.   The term "cycling", as used herein, means adjusting the temperature of the CST polymer gel through the CST range, whereby the gel becomes solvated, followed by desolvating, or vice versa, as the
20 CST is passed.

A ligand (binding pair) immobilized within the gel may be cycled through the gel CST to imbibe solution from an environment and bind a substance of interest. Further temperature cycling will expel unbound substance/
25 solution.   The ligand bound substance may then be released from the immobilizing binding pair component by further cycling the gel in an eluting solution that breaks either the bond to the polymer gel or the binding pair bond.   The substance of interest may then be expelled from the gel.
30 The eluting solution may effect the breaking of the bonds or linkage by a change in pH, the presence of certain ions and ionic concentrations, the presence of hydrogen bonders, certain solvents, water structurers, destructurers, oxidants or reductants, reactants, affinity ligands and the
35 like.

As noted above, a substance may be delivered to or removed from an environment by employing a ligand that

17

one may immobilize a drug, an antibody, or other biomole-
cule to the backbone of this gel via such linkages, and
keep the gel in a dry or shrunken state. When such a gel
is immersed in a solution below its LCST, it will swell and
5   imbibe solvent, and if the solvent has the capability of
degrading the linkage, then the drug (or antibody/antigen
complex) or other biomolecule will be released from the
backbone of the gel, and upon subsequent warming may be
released from the gel into the surrounding environment as
10   the gel collapses above its LCST.

The ligand immobilized (physically and/or
chemically) within the polymer gel may be a binding
component of an affinity binding pair. Suitable affinity
binding pairs include an antibody which binds with an
15   antigen or hapten of interest. A receptor may be bound to
the polymer gel that is designed to bind with a hormone,
vitamin, lectin, drug, dye or lipid binding partner in
solution. Other ligand binding pair components include
lectin and polysaccharide or glycoprotein; DNA, RNA
20   (single- or double- stranded) with complementary DNA, RNA
or oligonucleotides or proteins or steroids; ion with
chelator, ionophore, complexer; and stable-free radical
with free radicals.

Suitable ligands for immobilization within the
25   gel may also include a nonspecific binding component that
is suitable for reacting with a binding partner of interest
in an environment. For example, an anion or polyanion
could be incorporated or immobilized within the polymer gel
and bind, via ionic bonding, with a cation or polycation in
30   the environmental solution. An anion and cation pair in
the environmental solution could bind with a polyanion/
polycation complex within the gel. One may immobilize a
lipid or hydrophobic ligand within the polymer gel which
would bind via hydrophobic bonding with a lipophile in
35   solution. Acid/base-type interactions could be used to
affect binding by immobilizing an electron donor which

19

swollen gel to shrink noticeably and become somewhat hard
or brittle.   These mechanical and/or dimensional polymer
gel changes can be used to affect mechanical movements that
serve to signal a temperature change.   Conversely, a
5   temperature change can be used to affect mechanical or
dimensional changes.

Wettability of the surface of the CST polymer gel
changes as its temperature changes through the gel CST. An
LCST polymer gel loses wettability as the temperature is
10 raised through the LCST.   These changes are observable by
means of conventional contact angle measurements. In addi-
tion, the wettability phenomenon is important for surfaces
which are in contact with reactant solutions. Surfaces may
be wetted or dewetted as the temperature is varied above
15 the CST for a desired effect. For instance, the wetting or
dewetting could be used as a signal to transfer heat into
or out of the system, or could be used to eliminate fouling
components from a surface.

The gels of the present invention also change
20 optical characteristics at the CST. For example, increas-
ing a gel temperature above its LCST causes the gel to
become opaque.   If an enzyme is immobilized within a gel
and is included in a membrane system that is viewed
optically, a temperature increase to an undesirable level
25 could be detected by a change in the optical transmission
of the gel membrane. This change can be used to generate a
signal providing feedback to a coolant system that turns on
and off in response thereto.

The CST polymer gel materials may also be employed
30 in a variety of forms. For example, the materials may be
utilized as films or membranes, tubes, hollow fibers, solid
fibers, fabrics (woven, knit or non-woven), molded objects,
solid particles, capsules, polymeric micelles or liposome-
like structures. Likewise, they may be applied as coatings
35 on solid surfaces or in the pores of porous solids, as
solutions, particulate suspensions, etc. Coatings may be

21

biologic aggregates such as organelles and whole cells
themselves. Living cells contain many enzymes and can be
immobilized within LCST polymer gels such that when the
temperature is warmed above the LCST, the polymer gel will
5   shrink and squeeze out the fluid within the pores. This
aqueous solution could contain the product secreted from
the cells, and then would potentially enhance the recovery
of that product by this rapid delivery process. Addition-
ally, when the gel is reswollen below the LCST, this would
10   enhance the rate of mass transfer of nutrients, enzyme
substrates and other reactants, and oxygen to the cell,
which it requires in order to synthesize and secrete the
product of interest. Thus, a temperature cycling in such
an immobilized cell system could provide both enhanced
15   yields and enhanced rates of production of specific biologi-
cal products of enzymatic processes within living cells.
         The following examples are offered by way of
illustration and not by way of limitation.

20   EXAMPLE I
         A METHOD OF MAKING A THERMALLY REVERSIBLE GEL OF
             POLY-N-ISOPROPYL ACRYLAMIDE (NIPAAm)

         A solution of N-isopropyl acrylamide monomer in
25   dimethyl sulfoxide (DMSO) was prepared at various monomer
concentrations. Methylene bis-acrylamide (MBAAm) was added
as a cross-linker to the solution at various cross-linker
ratios of MBAAm/NIPAAm. Benzoyl peroxide (BP) was added at
a constant ratio of 0.001 mole BP/mole NIPAAm. The
30   solutions were sparged with N_2 and kept over N_2 at room
temperature. Then N,N dimethyl toluidene (NNDMT), a
co-catalyst with BP, was added dropwise with stirring until
the ratio of NNDMT/BP = 1 was reached. The solutions were
each then poured quickly between glass plates spaced 0.75
35   mm apart and sealed at the edges. The plates were immersed
in cool water and polymerization allowed to proceed 30-60
minutes. The plates were separated, the gels removed and

having a molecular weight of 17,800, and a like amount of
vitamin B_{12}, having a molecular weight of 1,350.   The
samples were incubated at 4°C overnight.   The films were
then removed from the original solution, quickly rinsed in

5   room temperature buffer and then deswelled in 10 ml of warm
buffer at 50°C for 4 minutes.

 Concentrations of the myoglobin and vitamin B_{12}
released were determined by absorption at 280 nm and 360
nm, respectively.   Figures 1 and 2 report the weight ratio

10   of myoglobin or vitamin B_{12} to buffer released, reported as
a function of cross-linking agent/monomer ratio densities.
Comparing polymer gels made with 20% NIPAAm monomer in
water with the 20% NIPAAm polymerized in DMSO shows that
the water-synthesized 20% NIPAAm polymer absorbed and

15   delivered myoglobin while the DMSO-synthesized 20% NIPAAm
gel did not.   Referring to Figure 2, vitamin B_{12}/buffer
release as a function of cross-linker to monomer ratio is
reported.   Figure 2 reports that both the 20% NIPAAm gels,
whether polymerized in DSMO or in water, absorbed and

20   delivered vitamin B_{12}.

 The comparison of the Figure 1 and 2 curves for
20% NIPAAm demonstrates that LCST hydrogels are capable of
distinguishing, with respect to absorption and delivery, on
the basis of molecular size.   The example demonstrates that

25   selection of important synthesis factors, such as cross-
linker to monomer ratio and synthesis solution composition,
may be used to affect the removal or delivery of molecules
or substances from or to the environment.

 Referring to Figure 3, the ratio of vitamin B_{12}

30   to buffer released on heating polyNIPAAm gels from 4°C to
50°C as a function of time is reported.   Release kinetics
of vitamin B_{12} from various gels show two regions over time.
The first region, occurring within the first five minutes
of the temperature change, is a relatively sudden release

35   of the solution nearest the surface of the gel and retained
within its pores.   The region thereafter shows a much
slower diffusion rate out of the gel after the initial

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content also has a significant influence on water content
at higher temperatures. As shown in Figure 4, some gels
have passed through their LCST, while others have not
reached their LCST.   It is possible that at high enough
5 comonomer contents the gel will not exhibit an LCST up to
100°C in water.

EXAMPLE IV

METHYLENE BLUE ABSORPTION AND DELIVERY AS A FUNCTION OF
10   METHACRYLICACID CONTENT OF NIPAAm POLYMER GELS

        Gel polymer samples were made in accordance with
Example III above, in which 0-5% methacrylic acid, in
separate samples at 1% increments, was copolymerized with
15 the poly-NIPAAm polymer. Methylene blue was absorbed into
those samples in order to determine delivery capabilities
as a function of amount of methacrylic acid incorporated
within the gel.
        The dried gels were equilibrated in a dye
20 solution of 1% methylene blue dissolved in a 50/50 methyl
alcohol and a 0.1 M Tris buffer (pH 8.61) solvent for 24
hours at room temperature. The polymer gel is dipped in a
buffer at pH 7.4 at 20°C for 1-2 seconds to wash off excess
solution on the surface. The gel is then placed in a
25 buffer to release the methylene blue at temperatures of 20,
30, 35, 40 and 50°C. The amount of methylene blue released
into the solution was determined using a UV-visible spectro-
photometer. The following table reports the methylene blue
initially absorbed into the gels.

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A comparison of the release rates of methylene
blue into aqueous buffer or distilled water from NIPAAm
polymer gels including 1% methacrylic acid was conducted.
The results, as depicted in Figure 6, show that including
5   buffer salts in the surrounding solution made it easier to
deliver methylene blue on heating above the LCST.   This
occurs because the salts break the negative/positive ionic
bond between the negatively charged methacrylic acid
component of the gel and the positively charged methylene
10   blue.   Thus, the buffer is acting as an eluting solvent.
This action is important in both release and recovery of
many binding partner solution components.   Other systems of
interest include, for example, assaying antigen in an
immunoassay or recovering a product such as a peptide
15   vaccine (which acts as an antigen to an immobilized
antibody), when using these gels for bioseparations.   The
first binding partner, e.g., antibody, may be immobilized
within the gel by including it in the polymerization
solution as the free binding partner, or as the monomer
20   conjugated binding partner.   The immobilized binding
partner may be any of the specific or nonspecific binding
partners, as listed above.

EXAMPLE V

25   ASPARAGINASE ENZYME ACTIVITY AFTER IMMOBILIZATION
WITHIN NIPAAm AND NIPAAm-METHACRYLIC ACID POLYMER GELS

In this procedure, 5 ml of 20% NIPAAm solution in
0.1 M Tris buffer (pH 8.6) were mixed with various percent-
30   ages of methacrylic acid, as noted above.   The solution was
combined with a MBAAm cross-linker in the ratio of one mole
of cross-linker per 750 moles NIPAAm.   To this solution, an
oxidizing redox catalyst component, comprising 10 micro-
liters of 10% ammonium persulfate solution in distilled
35   deionized water, was added.   The mixture was degassed under
vacuum and blanketed with nitrogen.   0.5 ml of asparaginase
enzyme solution in 0.1 M Tris buffer was added to the

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The data presented in Figures 7-10 show that the immobilized enzyme activity may be "turned off" by heating the polymer gel above its LCST, whether the enzyme is incorporated as free enzyme in the gel or as a monomer-
5 conjugated enzyme bound to the polymer gel during preparation of the gel. The curves show that the presence of 1% methacrylic acid shifts the LCST and thus the "turn off" temperature. Further, the data demonstrate that the "turn off" mechanism is reversible, i.e., that the enzyme retains
10 activity during a number of temperature cycles. The gels demonstrate that such reversibility is possible for some gels, such as NIPAAm containing 1% methacrylic acid, while not in others, such as NIPAAm containing no methacrylic acid. It appears that the enzyme may be denatured when the
15 NIPAAm gel is cycled to 50°C, but remains active when the gel includes a methacrylic acid component, possibly due to the higher water content of the methacrylic acid-containing gel and/or to the local pH within the gel. The temperature combined with the composition of the gel may act together
20 to denature the enzyme.

        Figure 11 shows the water content of polymer gels that include asparaginase, immobilized therein. These data show that the presence of enzyme in gels does not effect the water content versus temperature curves in a buffer.
25 The enzyme may be incorporated as free enzyme or be bound onto the backbone by its monomer conjugation during gel formation. A major and controlling factor demonstrated by these data is the impact of the monomer, in this example, methacrylic acid, and its concentration.
30

EXAMPLE VI
ASPARAGINASE ENZYME ACTIVITY AFTER IMMOBILIZATION
WITHIN NIPAAm AND AAm POLYMER GELS

        N-succinimidyl methacrylate (NSMA) dissolved in
35 dimethylformamide was combined with a solution of asparaginase in 0.1 M Tris buffer, pH 8.6, for a total monomer:enzyme ratio of 91.7:1. This mixture was allowed

31

Table: EXAMPLE VI

Sample Code	Relative Amount(%)	
	NIPAAm	AAm
NA-100	100	0
NA-95	95	5
NA-90	90	10
NA-85	85	15

Enzyme activity was measured at varied tempera-
tures between 20° and 60°. For these measurements, the
gels were first removed from storage at 4°C and equili-
brated at room temperature for 1 hour. The gels were
reacted for 10 minutes at room temperature, equilibrated at
30°C for 15 minutes, then reacted for 10 minutes in
asparagine solution at 30°C. This pattern of equilibration
for 15 minutes, then reaction for 10 minutes was repeated
for each temperature studied. The specific enzyme activi-
ties of the gels are shown as a function of increasing
temperature in Figure 12. It can be seen that the enzyme
activities parallel the water contents of the gels. Thus,
the activity of the immobilized catalyst (enzyme) may be
"shut off" by raising the temperature. Collapse of the gel
will both retard or eliminate diffusion of reactants (sub-
strate) into the gel as well as change the microenvironment
of the enzyme.

If the catalytic gel is to be useful for such
reaction control, it must act reversibly. The specific
enzyme activities of the gels were also studied by cycling
them between 30°C and 40°C. The results are shown in
Figure 13. It is evident that the enzyme activity is
reversible in all of these gels. In addition, the dramatic
drop in gel enzyme activity in going from 30° to 40° for
the NA-100 and NA-95 gels is in sharp contrast to the NA-90
and NA-85 gels, which show a rise in activity for the same
temperature changes. This is expected because the last two
gels are above their LCSTs at that temperature.

33

immersed wet into 37° or 50° water, the release kinetics
show two linear regions, a fast rate followed by a slow
rate. Only when the gel is initially dry was one linear
release rate observed. Therefore, thermally reversible
5 homopolymer gels, such as lightly cross-linked poly NIPAAm,
can be employed for fabricating matrix drug delivery
systems which display zero-order release.

                    EXAMPLE VIII
10   GRAFTED SURFACES FOR MINIMIZING PROTEIN AND CELL ADHESION

          N-isopropyl acrylamide and its copolymers with
acrylamide were radiation grafted to a silicone rubber
substrate. The silicone rubber (SR) substrate material
15 used was 20 mil Silastic film (Dow Corning 500-5). The
monomers employed were NIPAAm and AAm and were used as
received without further purification. Irradiations were
carried out in a Cobalt -60 source with the films immersed
in aqueous solutions of the monomers. Cupric nitrate was
20 added to inhibit homopolymerization in the solution. In a
few cases, nitrogen atmosphere was used, but in most of the
studies an air atmosphere was present. After irradiation,
each film was washed in deionized water overnight, dried in
a dessicator, and weighed to permit calculation of percent
25 grafting as 100 x (weight of grafted film - original film
weight + original film weight). Water contents of the
grafted films were measured at different temperatures after
equilibration at each temperature overnight, followed by
weighing the wet film, then drying and reweighing it in the
30 dry state. The water contents are reported as 100 x (wet
weight of grafted film - dry weight of grafted film) + (wet
weight of grafted film - original weight of film).
          Based upon empirical studies, the following exper-
imental conditions were selected for radiation grafting:
35 100 mmol/1 of Cu(NO_3)_2, 10% total monomer concentration
(NIPAAm or AAm or mixtures), air atmosphere, and an irradi-

35

as anticipated, the crossover at lower temperatures dis-
appears above the LCST region (31°-33°) of poly NIPAAm.

Grafting of hydrogels onto hydrophobic substrates
can yield more biocompatible surfaces while retaining the
5   desirable physical properties of the substrate material.
The rationale for grafting hydrogels is based on the
hypothesis that the more hydrophilic the polymer surface,
the lower the interfacial energy in the aqueous biological
environment and thus the lower the thermodynamic driving
10 force for protein adsorption and cell adhesion.   When
polymers are radiation grafted with gradually varying
mixtures of hydrophilic and hydrophobic monomers, protein
adsorption and cell adhesion often exhibit a minimum at
some intermediate graft copolymer composition.
15

From the foregoing it will be appreciated that,
although specific embodiments of the invention have been
described herein for purposes of illustration, various
modifications may be made without deviating from the spirit
20 and scope of the invention. Accordingly, the invention is
not limited except as by the appended claims.

25

30

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7. The method of claim 2 including, after the step of separating, contacting the polymer gel/substance with a solution capable of releasing the bound complex from the polymer gel.

8. The method of claim 7 wherein said solution contains a composition selected from the group consisting of added acids and bases, and enzymes.

9. The method of claim 1 wherein said binding component specifically binds with said substance.

10. The method of claim 9 wherein said binding component is a receptor and said substance is selected from the group consisting of hormones, vitamins, lectins, drugs, dyes, and lipids.

11. The method of claim 9 wherein said binding component is an antibody and said substance is an antigen.

12. The method of claim 9 wherein said binding component is an enzyme and said desired substance is a substrate, inhibitor, coenzyme or cofactor.

13. The method of claim 9 wherein said binding component is selected from the group consisting of lectins, RNA, DNA (single or double stranded), ions, and stable free radicals and said desired substance is selected from the group consisting of polysaccharides, glycoproteins, RNA or DNA complementary with said binding component, oligonucleotides, proteins, steroids, chelators, complexers, ionophores, and free radicals.

14. The method of claim 1 wherein said binding component binds said desired substance nonspecifically.

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22.   The method of claim 1 wherein said binding component is immobilized to said polymer gel via a spacer molecule.

23.   The method of claim 1 wherein said binding component is immobilized to said polymer gel by conjugating said binding component to a monomer and subsequently copolymerizing with additional monomers and cross-linking agents.

24.   A method for separating a desired substance from a solution, comprising:
        introducing a polymer gel that is characterized by a critical solution temperature and having a pore structure adapted to selectively retain said desired substance within the gel on the basis of size into a solution containing said desired substance; and
        adjusting the temperature of the polymer gel/solution, thereby causing said gel to incorporate the desired substance and separate it from the solution.
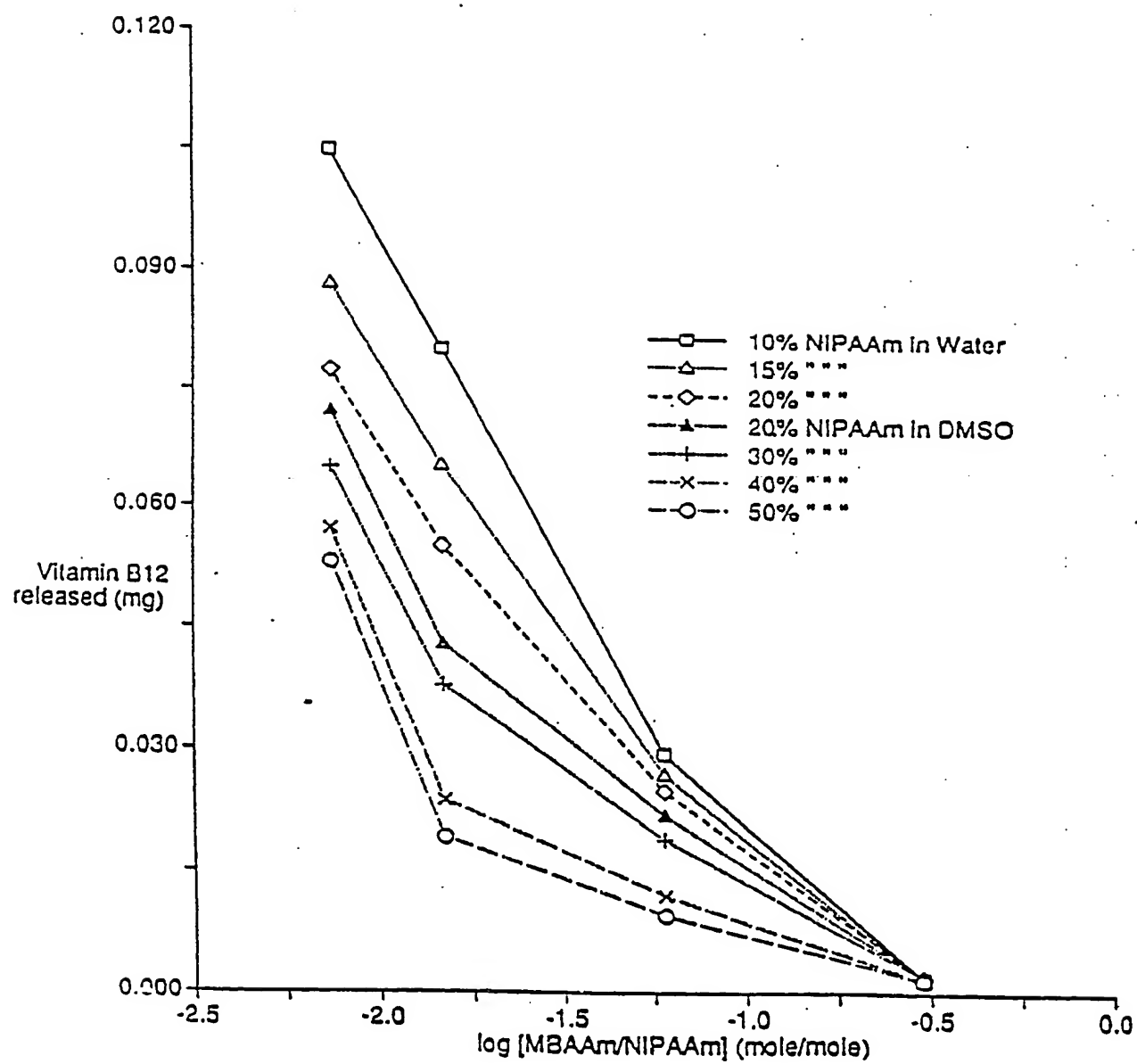
25.   The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of cross-linking agent employed with the polymer to form the polymer gel.

26.   The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of monomer employed to form the polymer gel.

27.   The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of solvent employed to form the polymer gel.
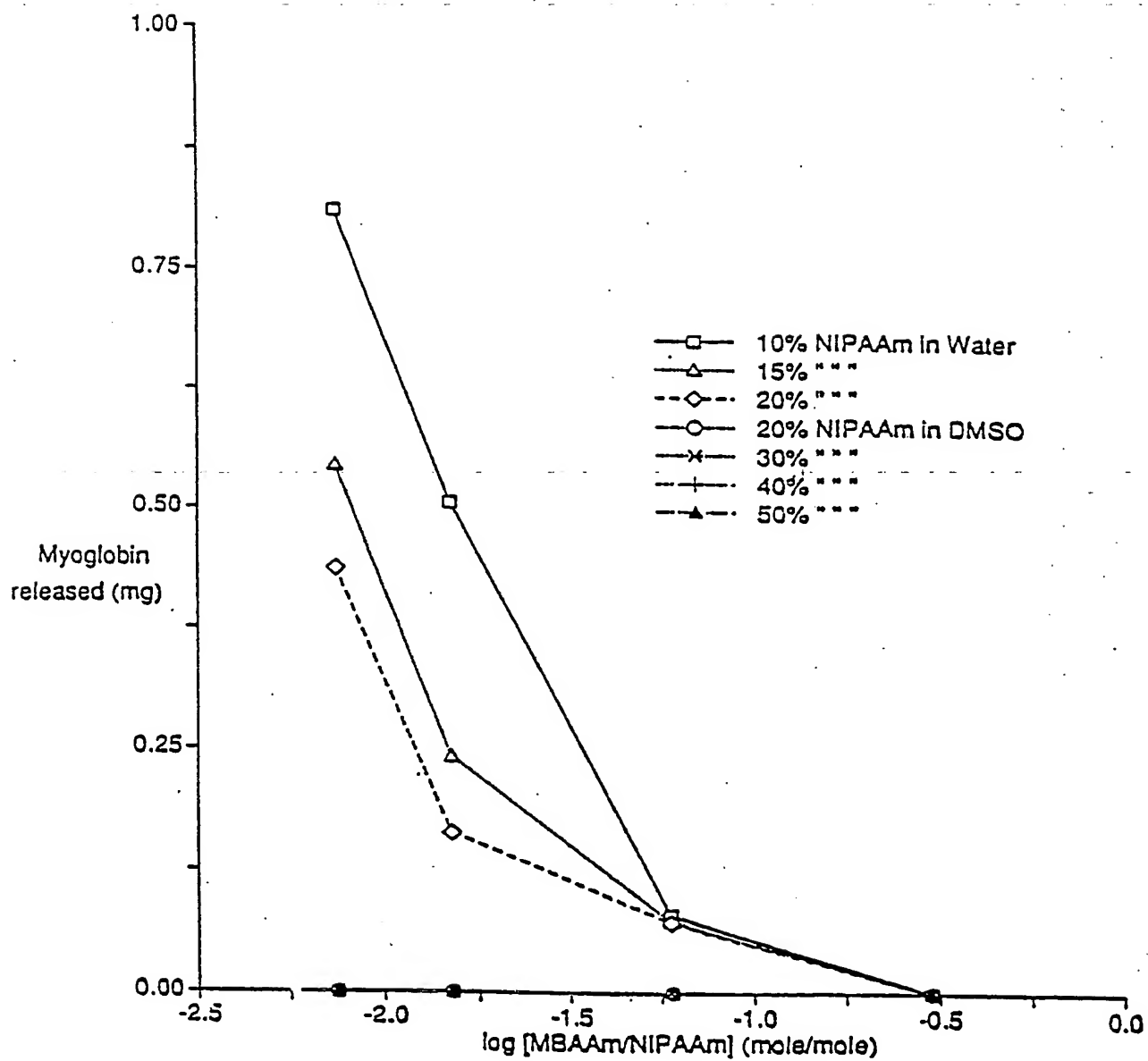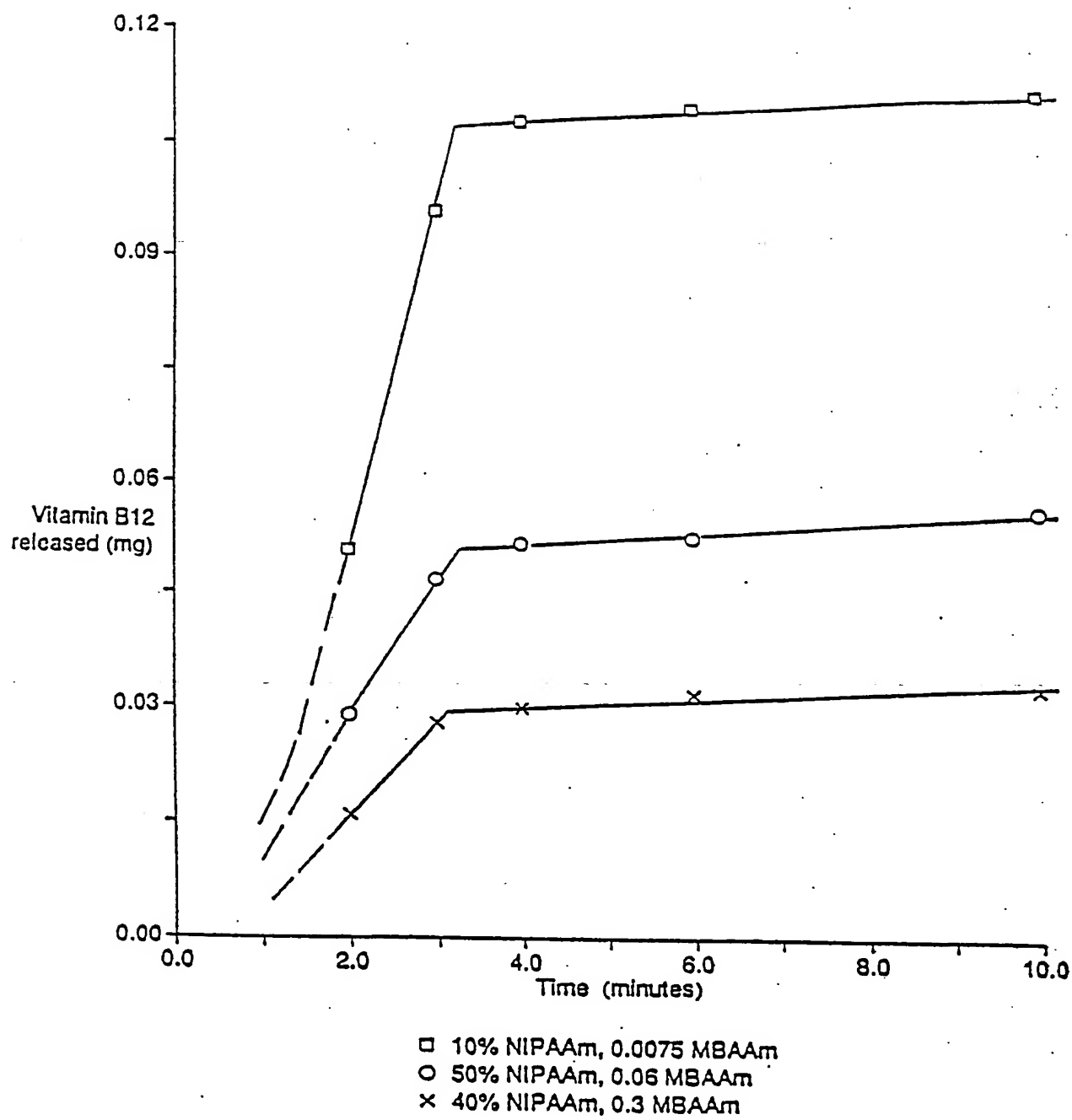
41

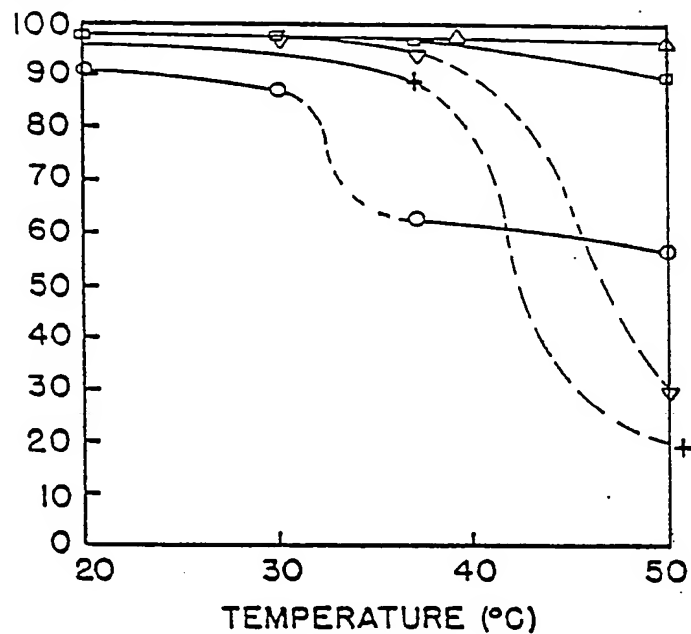hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and copolymers thereof.

36. The method of claim 24 wherein said polymer is characterized by an upper critical solution temperature (UCST).

37. The method of claim 36 wherein the UCST polymer gel includes a polymer selected from the group consisting of polyacrylic acid and polyvinyl alcohol.

38. A method for delivering a substance into a selected environment, comprising:
    incorporating a desired substance into a polymer gel characterized by a critical solution temperature by binding said substance to a binding component immobilized within said gel; and
    introducing said polymer gel/substance into a selected environment containing one or more agents capable of releasing said substance from said binding component, thereby delivering said substance into the environment.

39. The method of claim 38 including, subsequent to the step of introducing, adjusting the temperature of the polymer gel to cause the gel to actively deliver the desired substance into the environment.

40. The method of claim 38 wherein said agent is selected from the group consisting of acids or bases, salts, ionic and non-ionic detergents, organic solvents, and chaotropic agents.

41. The method of claim 38 wherein said agent is a compound capable of forming a stronger complex with the desired substance than the binding component.

42. The method of claim 38 wherein said binding component specifically binds with said substance.

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49. The method of claim 48 wherein said agent is selected from the group consisting of acids or bases and enzymes.

50. A method for selectively delivering a desired substance into an environment, comprising:

incorporating a desired substance into a polymer gel characterized by a critical solution temperature and having a pore structure adapted to selectively retain said desired substance within the gel on the basis of size;

introducing the polymer gel/substance into a selected environment; and

adjusting the temperature of the polymer gel/ substance to selectively deliver the desired substance into the environment.

51. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of cross-linking agent employed with the polymer to form the polymer gel.

52. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of monomer employed to form the polymer gel.

53. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of solvent employed to form the polymer gel.

54. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the amount and/or composition of initiators employed to form the polymer gel.

55. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of chain transfer agents employed to form the polymer gel.

FIG. 1

FIG. 2

Legend:
- □ 10% NIPAAm in Water
- △ 15% " " "
- ◇ 20% " " "
- ○ 20% NIPAAm in DMSO
- ✕ 30% " " "
- + 40% " " "
- ▲ 50% " " "

Y-axis: Myoglobin released (mg)

X-axis: log [MBAAm/NIPAAm] (mole/mole)

FIG. 3

EFFECT OF TEMPERATURE ON WATER CONTENTS OF LOST HYDROGELS

FIG.4

O:MN-O; +:MN-I; ▽:MN-2; □:MN-3; △:MN-5

FIG. 5A

FIG. 5B

% Dye Released into Buffer

Time (hr.)

20°
50°
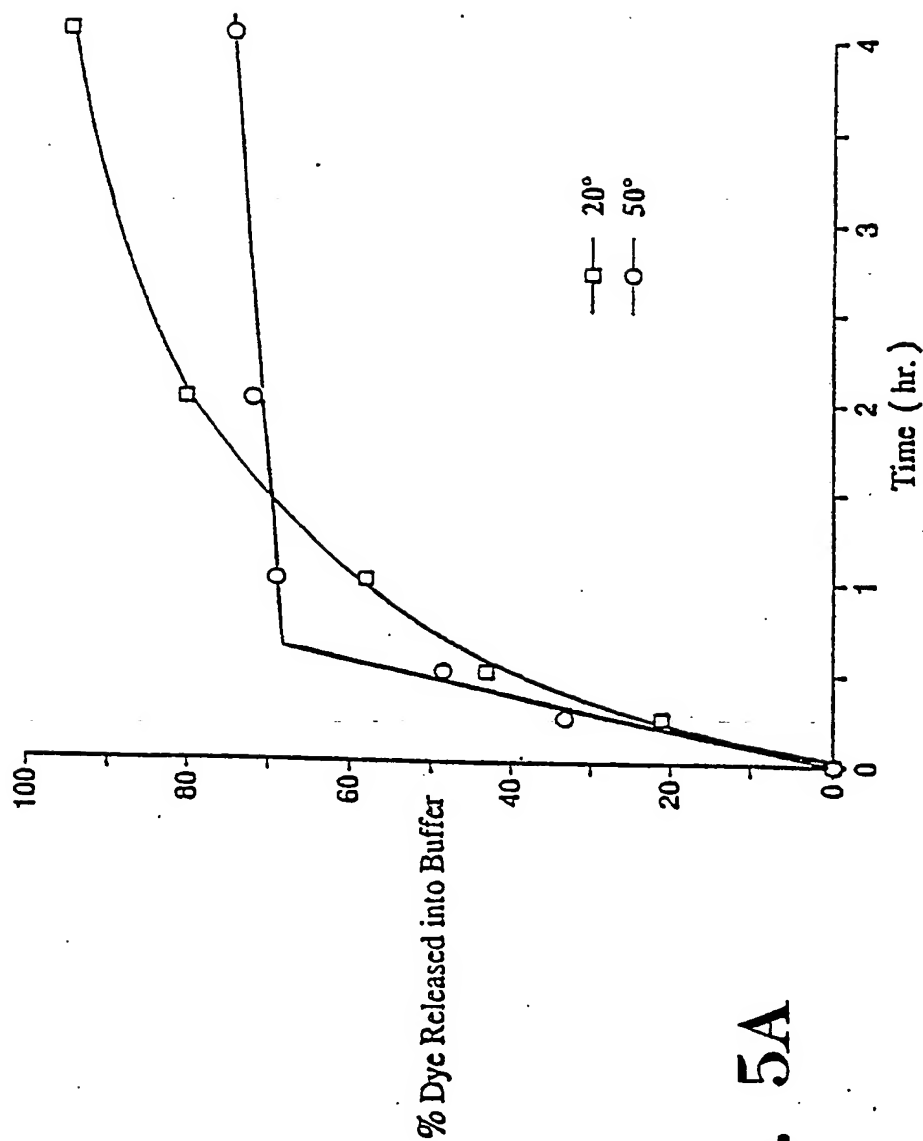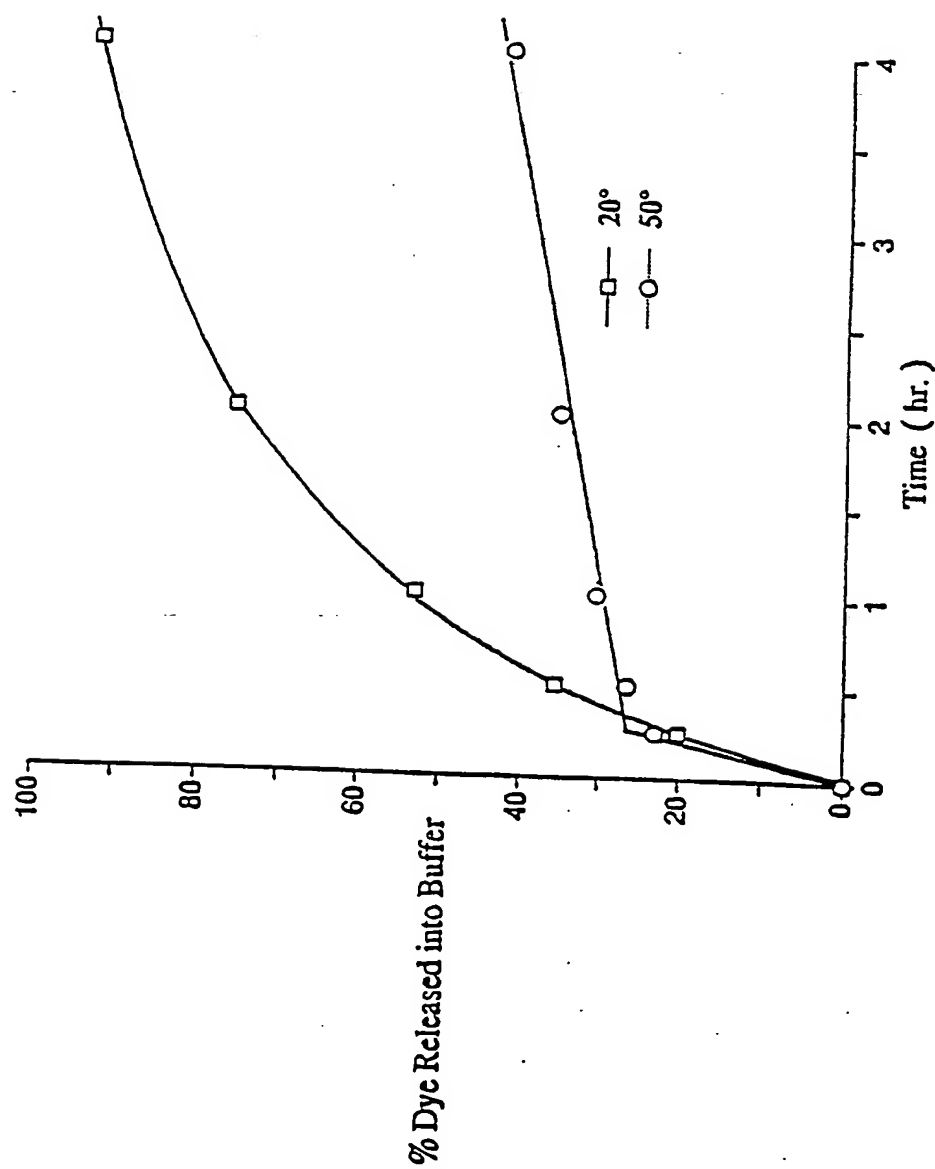
FIG. 5C
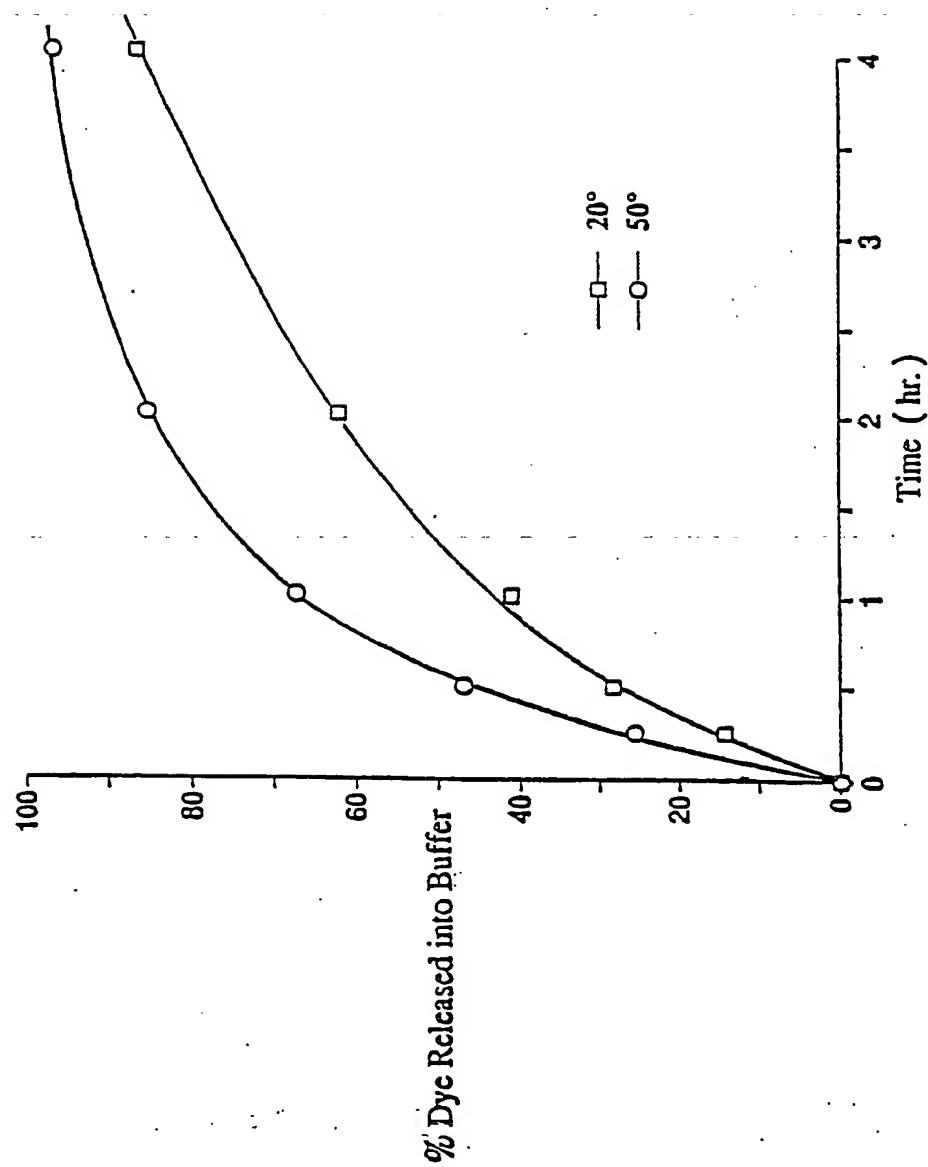
TEMPERATURE DEPENDENCE OF RELEASE RATE OF METHLENE BLUE
FROM MN-I GELS IN DIFFERENT MEDIA

ONE HOUR RATE (% DYE RELEASED / HR.)
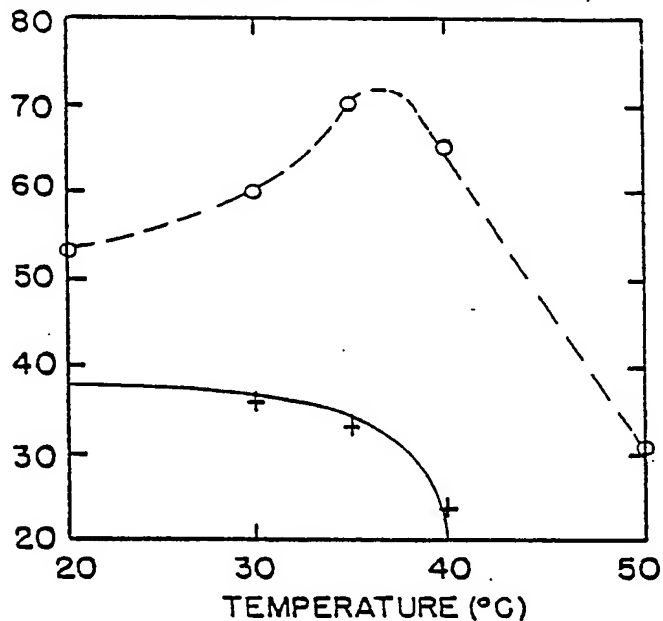
O: BUFFER; + : D. I. H$_2$O.

FIG.6

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY
(FIRST RUN)

SPECIFIC ACTIVITY(I.U./g. POLYMER
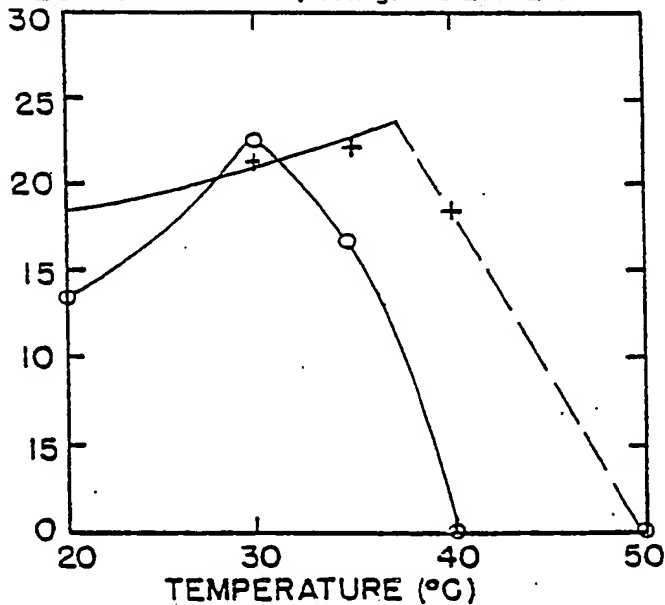
FIG.7

O: MN-O-f; + : MN-I-f.

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY(THIRD RUN)
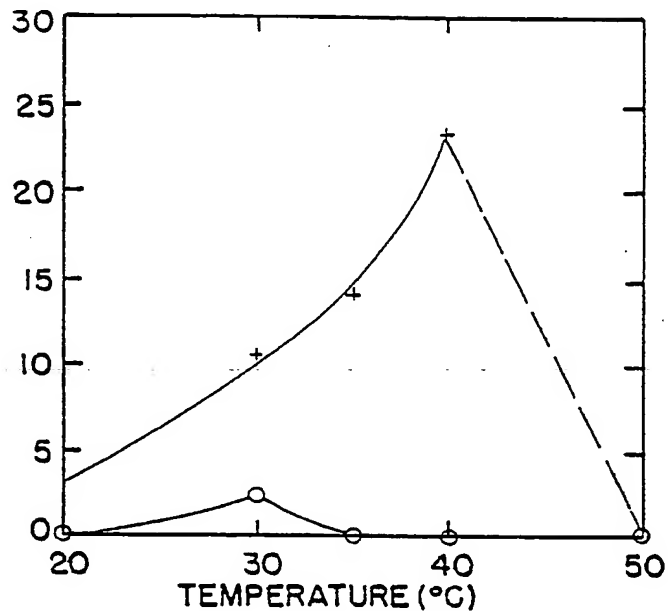
SPECIFIC ACTIVITY (I.U./g. POLYMER)
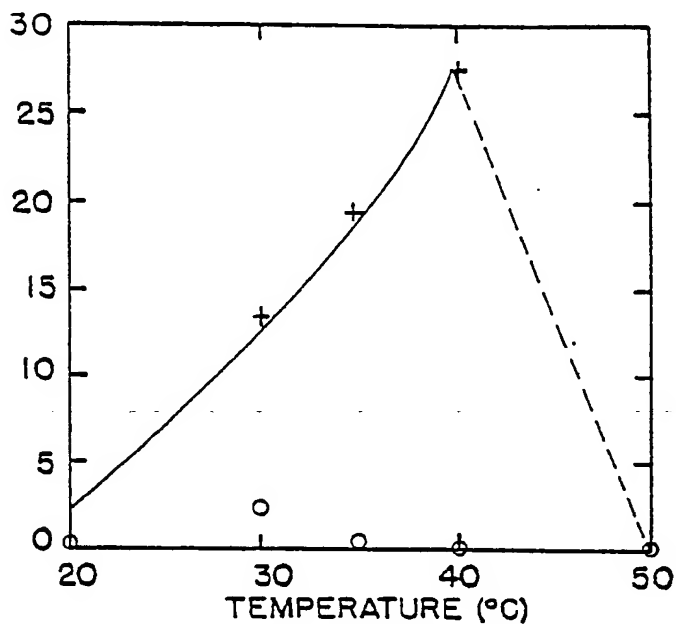
FIG. 8

O: MN-O-f, +: MN-I-f

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY (FIRST RUN)

SPECIFIC ACTIVITY (I.U./g. POLYMER)
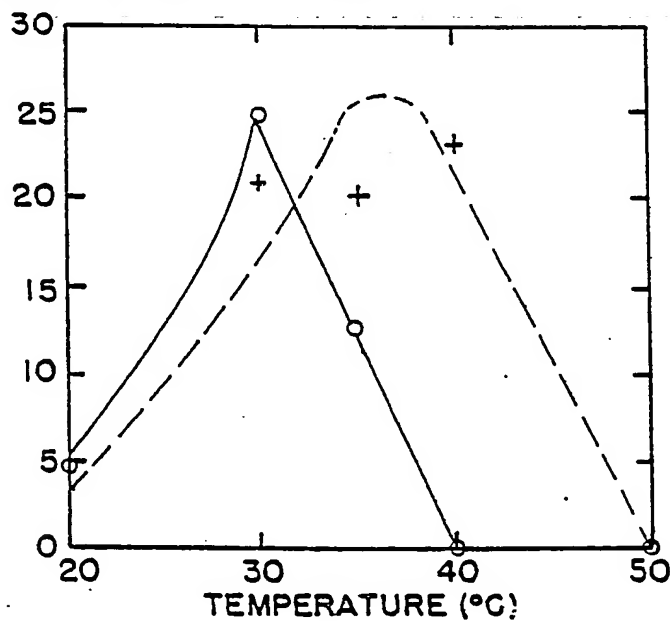
FIG. 9

O: MN-O-C, + MN-I-C.

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY (THIRD RUN)

SPECIFIC ACTIVITY (I.U./g. POLYMER)

FIG. 10

O:MN-O-C;+:MN-I-C.

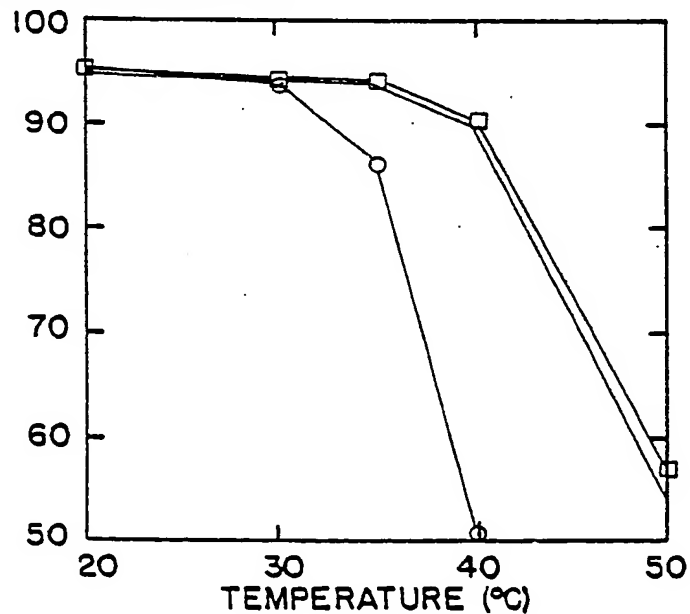TEMPERATURE DEPENDENCE OF WATER CONTENT OF ASPARAGINAS, -IMMOBILIZED DISCS
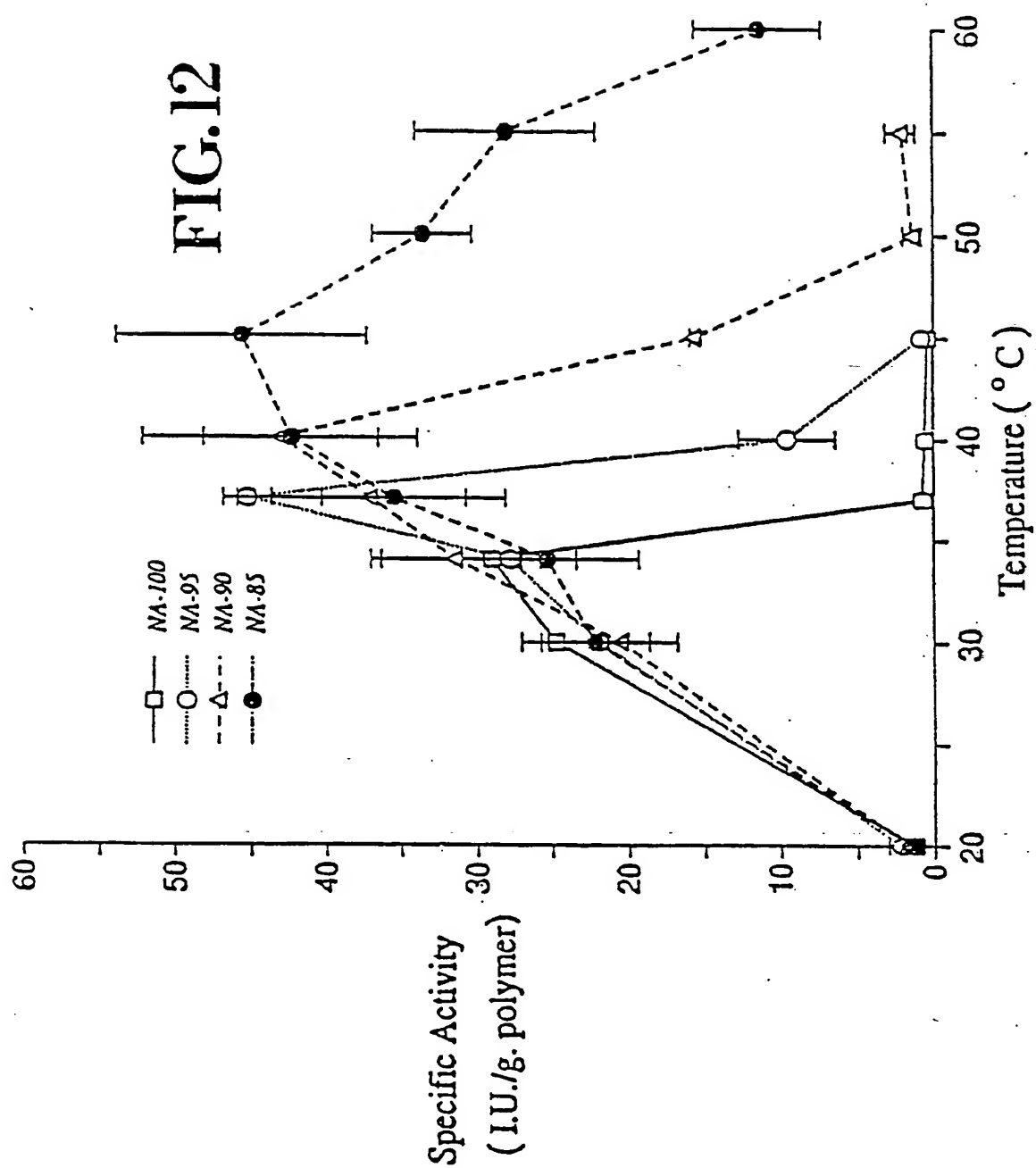
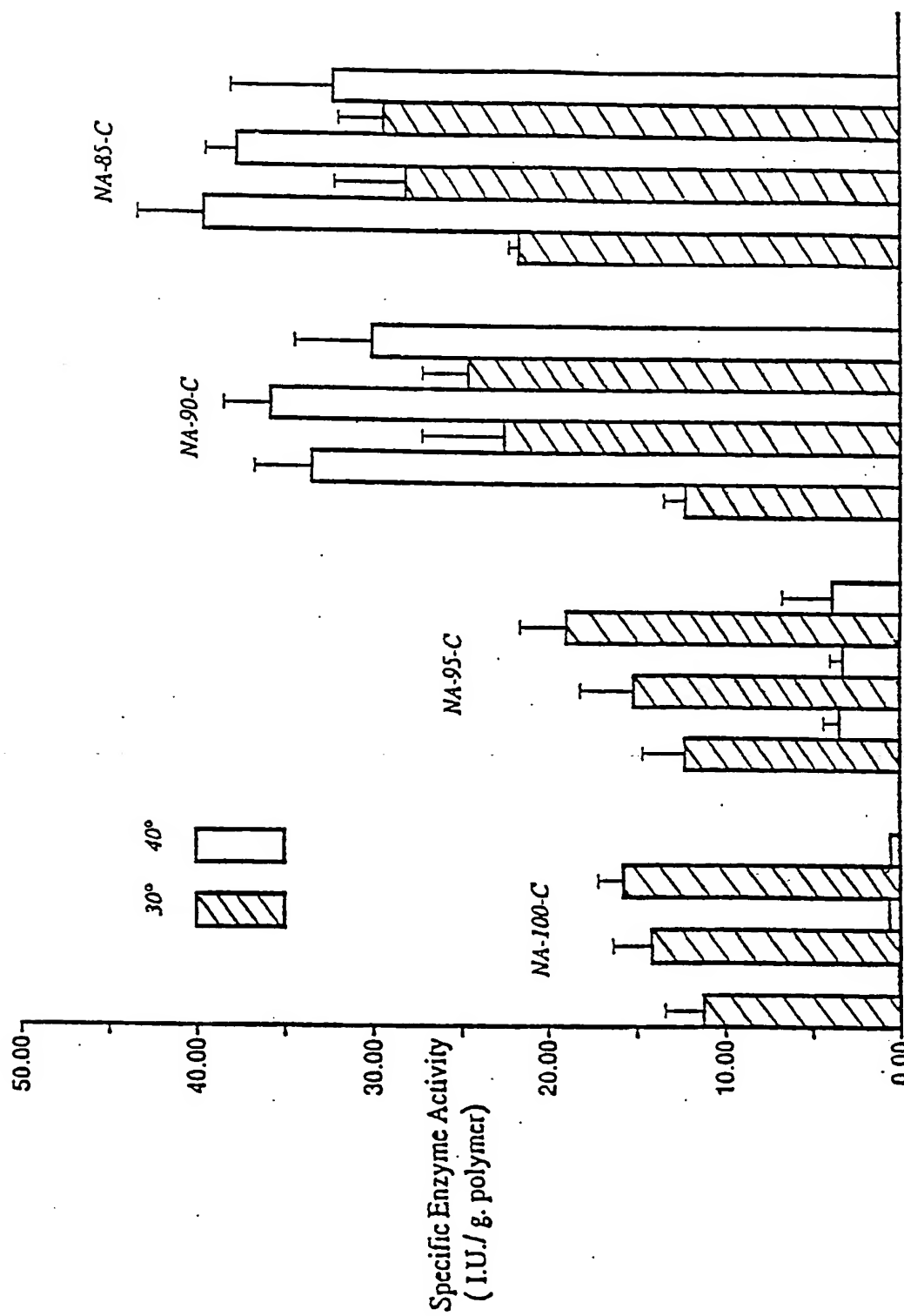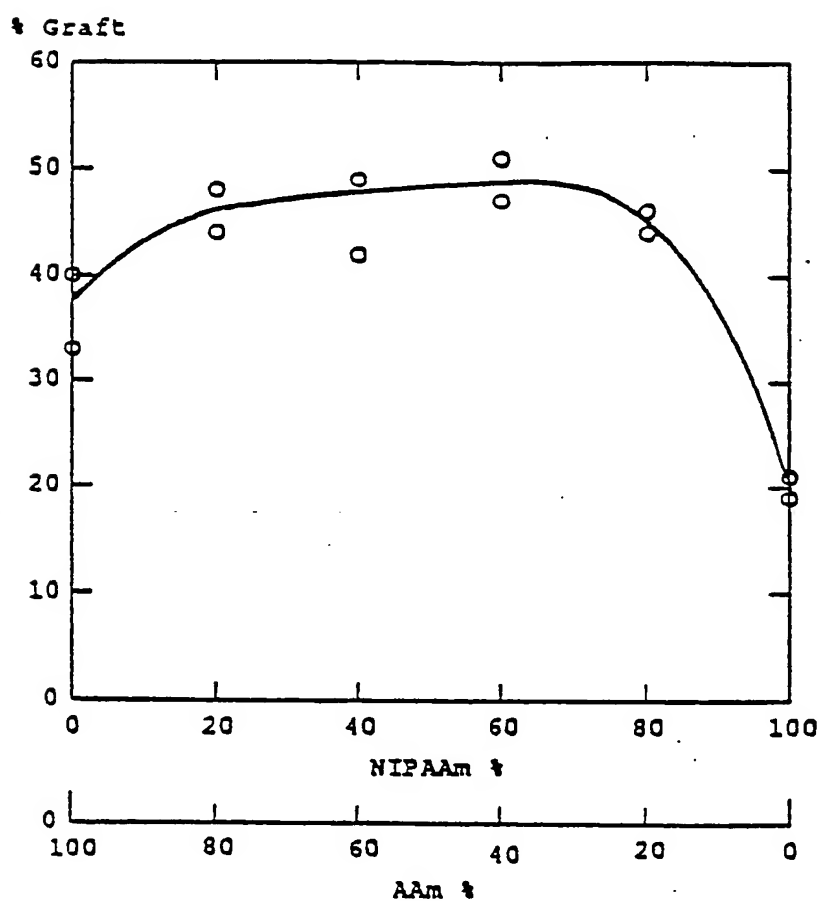WATER CONTENT (%)

FIG.11

O:MN-O-C;+:MN-I-C;▽:MN-O-f;□:MN-I-f

FIG.12

FIG.13

FIG.14

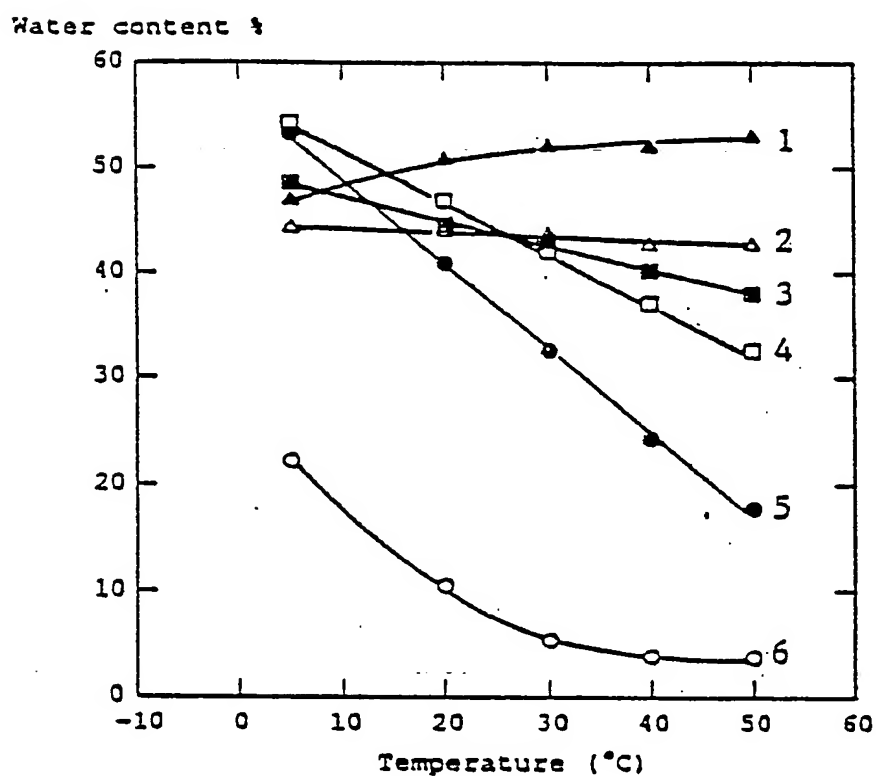## Grafting NIPAAm/AAm Mixtures to Silastic

Dose: 0.68 Mrad; air atmosphere
Solutions: 100 mM Cu(NO₃)₂ in D.I. water
+ 10% (wt) total monomer

-14/14-

Effect of Temperature on
Water Contents of Silicone Rubber
Films Grafted by NIPAAm/AAm Mixtures

Dose: 0.68 Mrad; air atmosphere.
Solutions: 100mM $Cu(NO_3)_2$ in D.I. H_2O.
+ 10% (wt) total monomer

FIG.15

Film no.	NIPAAm/AAm
1	0/10
2	2/8
3	4/6
4	6/4
5	8/2
6	10/0

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4:   B 01 D 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC^4	B 01 D

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	GB, A, 2149803 (MITSUI TOATSU CHEMICALS) 19 June 1985 see page 1, line 50 - page 7, line 38; pages 17-18, claims 1-22	24-35,50-56
Y		1,2
	--	
Y	US, A, 3414509 (BLOCH) 3 December 1968 see column 4, line 31 - column 11, line 63	1,2
A		4,7,14,15, 38,39,48
	--	
A	EP, A, 0059598 (UNILEVER) 8 September 1982 see page 3, line 4 - page 7	4,5,9,11
	--	
Y	US, A, 4555344 (CUSSLER) 26 November 1985 see columns 5-6, claims 1-10; column 2, line 35 - column 7, line 43	24,25,32, 34
A	cited in the application	35
	--	
P,Y	Chemical & Engineering News, volume 64, no. 18, May 1986, (Washington, US), "Separation process uses swellable ./.	

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th July 1987	14 SEP 1987
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	M. VAN MOL

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO.   PCT/US 87/00886 (SA   17098)
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This Annex lists the patent family members relating to the
patent documents cited in the above-mentioned international
search report. The members are as contained in the European
Patent Office EDP file on 05/08/87

The European Patent Office is in no way liable for these
particulars which are merely given for the purpose of
information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A- 2149803	19/06/85	FR-A-	2553678	26/04/85
		NL-A-	8403204	17/05/85
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		JP-A-	60250017	10/12/85
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US-A- 3414509		None		
EP-A- 0059598	08/09/82	WO-A-	8202818	02/09/82
		AU-A-	8142982	14/09/82
		US-A-	4490290	25/12/84
		CA-A-	1188612	11/06/85
		AU-B-	558931	12/02/87
US-A- 4555344	26/11/85	None		
WO-A- 8606492	06/11/86	AU-A-	5909986	18/11/86
		EP-A-	0221175	13/05/87